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Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination —

Part 5: Specific rules for the preparation of milk and milk products

Microbiologie de la chaîne alimentaire — Préparation des échantillons, de la suspension mère et des dilutions décimales en vue de l'examen microbiologique —

Partie 5: Règles spécifiques pour la préparation du lait et des produits laitiers

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 463, *Microbiology of the food chain*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This second edition cancels and replaces the first edition (ISO 6887-5:2010), which has been technically revised. The main changes compared with the previous edition are as follows:

— the document has been aligned with ISO 6887-1, ISO 6887-2, ISO 6887-3 and ISO 6887-4;

— cross references have been added to ISO 6887-1 where relevant.

A list of all parts in the ISO 6887 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination —

Part 5: Specific rules for the preparation of milk and milk products

WARNING — The use of this document can involve hazardous materials, operations and equipment. It is the responsibility of the user of this document to establish appropriate safety and health practices and to determine the applicability of regulatory limitations before use.

1 Scope

This document specifies rules for the preparation of samples of milk and milk products and their suspensions for microbiological examination when the samples require a different preparation from the general methods specified in ISO 6887-1.

This document excludes the preparation of samples for both enumeration and detection test methods where preparation details are specified in the relevant International Standards.

This document is intended to be used in conjunction with ISO 6887-1.

This document is applicable to:

a) milk and liquid milk products;

b) dehydrated milk products;

c) cheese and cheese products;

d) casein and caseinates;

e) butter;

f) milk-based ice-cream;

g) milk-based custard, desserts and sweet cream;

h) fermented milks, yogurt, probiotics milk products and sour cream;

i) dehydrated milk-based infant foods, with or without probiotics.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 6887-1, *Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions*

ISO 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

ISO 11133, *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 6887-1 apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

4 Principle

The general principles for sample preparation and subsequent steps are detailed in ISO 6887-1. This document describes specific sample preparation for milk and milk products.

5 Diluents

5.1 List of diluents

Follow current laboratory practices as specified in ISO 7218. The composition of culture media and reagents and their preparation are specified in ISO 6887-1 or in the following procedures.

5.1.1 Basic materials. See ISO 6887-1.

5.1.2 Diluents for general use. Peptone salt solution, buffered peptone water and double-strength buffered peptone water are described in ISO 6887-1.

5.1.2.1 Quarter-strength Ringer's solution.

5.1.2.1.1 Composition

Sodium chloride (NaCl) (CAS No. 7647-14-5)	2,25 g
Potassium chloride (KCl) (CAS No. 7447-40-7)	0,105 g
Calcium chloride anhydrous ($CaCl_2$) (CAS No. 10043-52-4)	0,06 g[a]
Sodium hydrogen carbonate ($NaHCO_3$) (Cas No. 144-55-8)	0,05 g
Water	1 000 ml

[a] Alternatively, use 0,12 g of $CaCl_2 \cdot 6H_2O$ (CAS No. 10035-04-8).

5.1.2.1.2 Preparation

Dissolve the salts in the water. Adjust the pH, if necessary, so that after sterilization it is 6,9 ± 0,2 at 25 °C.

5.1.2.2 Peptone solution.

5.1.2.2.1 Composition

Enzymatic digest of casein	1,0 g
Water	1 000 ml

5.1.2.2.2 Preparation

Dissolve the peptone in the water. Adjust the pH, if necessary, so that after sterilization it is 7,0 ± 0,2 at 25 °C.

5.1.2.3 Phosphate buffer solution.

5.1.2.3.1 Composition

Potassium dihydrogen phosphate (anhydrous) (KH_2PO_4) (CAS No. 7778-77-0)	42,5 g
Water	1 000 ml

5.1.2.3.2 Preparation

Dissolve the salt in 500 ml of water. Adjust the pH, if necessary, so that after sterilization it is 7,2 ± 0,2 at 25 °C. Dilute to 1 000 ml with the remaining water.

Store the stock solution under refrigerated conditions.

Add 1 ml of this stock solution to 1 000 ml of water for use as diluent.

5.1.3 Diluents for special purposes. These diluents shall only be used for the preparation of initial suspensions.

5.1.3.1 Sodium citrate solution.

5.1.3.1.1 Composition

Trisodium citrate dihydrate ($Na_3C_6H_5O_7{\cdot}2H_2O$) (CAS No. 6132-04-3)	20,0 g
Water	1 000 ml

5.1.3.1.2 Preparation

Dissolve the salt in water by heating, if necessary, on a hotplate (6.3) at a temperature between 45 °C and 50 °C. Adjust the pH, if necessary, so that after sterilization it is 7,5 ± 0,2 at 25 °C.

5.1.3.1.3 Application

This solution is used for cheese and (roller-)dried milk, and some caseinates.

5.1.3.2 Dipotassium hydrogen phosphate solution.

5.1.3.2.1 Composition

Dipotassium hydrogen phosphate (K_2HPO_4) (CAS No. 7758-11-4)	20,0 g
Water	1 000 ml

5.1.3.2.2 Preparation

Dissolve the salt in the water by heating, if necessary, on a hotplate (6.3) at a temperature between 45 °C and 50 °C. For acid whey powder, adjust the pH so that for the primary dilution after sterilization it is 8,4 ± 0,2 at 25 °C. For cheese, roller-dried milk, fermented milk, yogurt, caseinates and sour cream, adjust the pH so that after sterilization it is 7,5 ± 0,2 at 25 °C.

5.1.3.2.3 Application

This solution is used for cheese, (roller-)dried milk, fermented milk, yogurt, some caseinates, dehydrated acid whey, and sour cream.

5.1.3.3 Dipotassium hydrogen phosphate solution with antifoam agent.

5.1.3.3.1 Composition

5.1.3.3.1.1 Dipotassium hydrogen phosphate solution

Prepare according to 5.1.3.2.

5.1.3.3.1.2 Antifoam stock solution

5.1.3.3.1.2.1 Composition

Polyethylene glycol 2000 (CAS No. 25322-68-3)	1 g
Water	100 ml

5.1.3.3.1.2.2 Preparation

Dissolve the polyethylene glycol 2000 in the water by mixing.

5.1.3.3.2 Preparation

Add 1 ml of the antifoam stock solution (5.1.3.3.1.2) to 1 l of the K_2HPO_4 solution (5.1.3.3.1.1). Adjust the pH so that for the primary dilution of both acid and lactic casein, after sterilization, it is 8,4 ± 0,2 at 25 °C, and for rennet casein, after sterilization, it is 7,5 ± 0,2 at 25 °C.

5.1.3.3.3 Application

This solution is used for acid casein, lactic casein and rennet caseins.

5.1.3.4 Tripolyphosphate solution.

5.1.3.4.1 Composition

Sodium tripolyphosphate pentabasic ($Na_5O_{10}P_3$) (CAS No. 7758-29-4)	20,0 g
Water	1 000 ml

5.1.3.4.2 Preparation

Dissolve the salt in the water by heating slightly on a hotplate (6.3), if necessary. The solution may be stored at a temperature of 5 °C ± 3 °C for a maximum of one month.

5.1.3.4.3 Application

This solution is used as alternative diluent for rennet caseins that are difficult to dissolve.

5.1.3.5 Diluent for general use with α-amylase solution.

5.1.3.5.1 Preparation

For a 25 g test portion, add 12,5 mg of α-amylase (EC 3.2.1.1, see Reference [1]) with a specific activity of approximately 400 units (= 6,7 µkat) per milligram to 225 ml of the diluent for general use (5.1.2). Use amounts in the same proportion for preparation of other test portions (e.g. for a 10 g test portion, add 5 mg of α-amylase to 90 ml of the diluent for general use).

NOTE The unit (often called the "international unit" or "standard unit") is defined as the amount of enzyme that catalyses the transformation of 1 µmol of substrate per minute under standard conditions.

5.1.3.5.2 Application

This solution may be used for foods containing starch, when the primary dilution presents a solubility problem.

NOTE An example of a solubility issue is when the initial suspension is too thick to mix or pipette.

5.2 Distribution and sterilization of the diluents

Follow ISO 6887-1.

5.3 Performance testing for diluents

For performance testing of all diluents, follow the procedures as specified in ISO 11133 and as described in Table 1.

Table 1 — Performance testing for diluents

Media	Function	Incubation	Control strains	WDCM numbers[a]	Reference medium	Method of control	Criteria
All diluents in this document	Diluent	45 min to 1 h (18 to 27) °C	*Escherichia coli*[c] *Staphylococcus aureus*	00012 or 00013 00034[b]	TSA (Tryptone soy agar)	Quantitative	±30 % of the enumeration at T_0 (±30 % of original count)

[a] Make reference to the reference strain catalogue available on http://www.wfcc.info for information on culture collection strain numbers and contact details.

[b] Strain to be used as a minimum.

[c] Strain free of choice. One of the strains shall be used as a minimum.

6 Apparatus

Usual microbiological laboratory equipment for general use (see ISO 7218 and ISO 6887-1) and, in particular, the following.

6.1 Water baths, capable of maintaining temperatures of (34 to 38) °C and (44 to 47) °C.

6.2 Spatulas or **glass rods**.

6.3 Hotplate or other apparatus, capable of gentle heating (not gas burners), and capable of operating at the required temperature.

6.4 Glass beads, of diameter about 6 mm.

7 Sampling

Sampling is not part of the method specified in this document. Follow the specific ISO document dealing with the product concerned. If there is no specific ISO document dealing with the sampling of the product concerned, it is recommended that the parties concerned come to an agreement on this subject.

Recommended sampling techniques are given in ISO 707 | IDF 50.

8 General procedures

8.1 General

All preparations and manipulations should be carried out using an aseptic technique with sterile equipment to prevent microbial contamination of samples from all external sources (see ISO 7218).

Follow the general procedure for preparation of the initial suspension as described in ISO 6887-1.

8.2 Frozen products

Follow ISO 6887-1.

8.3 Hard and dry products

To mix hard products in a peristaltic blender, place the sample and diluent in double- or triple-layered sterile bags to prevent puncturing and possible sample spillage, or alternately homogenize using a rotary blender when appropriate for hard, low moisture products.

Follow ISO 6887-1 for alternative preparation methods.

8.4 Liquid and non-viscous products

Follow ISO 6887-1.

8.5 Multi-component products

Follow ISO 6887-1.

8.6 Acidic products

Follow ISO 6887-1.

8.7 High-fat foods (fat content > 20 % mass fraction)

Follow ISO 6887-1.

9 Specific procedures

9.1 Milk and liquid milk products

Mix the test sample thoroughly so that the microorganisms are distributed as evenly as possible by rapidly inverting the sample container 25 times. Avoid foaming or allow any foam to disperse. The interval between mixing and removing the test portion shall not exceed 3 min.

Remove the test sample with a sterile pipette and prepare further dilutions in accordance with ISO 6887-1 or inoculate directly a medium or a broth in accordance with the procedure of the specific method of enumeration or detection.

9.2 Dehydrated milk, dehydrated sweet whey, dehydrated acid whey, dehydrated buttermilk and lactose

Thoroughly mix the contents of the closed container by repeatedly shaking and inverting it.

If the test sample is in the original unopened container and this is too full to permit thorough mixing, transfer it to a larger container, then mix. Open the container, remove the test portion required with a spatula (6.2) and proceed as indicated below. Immediately close the container again.

Prepare the initial suspension in accordance with ISO 6887-1 for dehydrated products and other low-moisture products, with a diluent for general use (5.1.2). For dehydrated acid whey, use dipotassium hydrogen phosphate solution (5.1.3.2) at pH 8,4 ± 0,2 or, if necessary, for roller-dried milk use sodium citrate solution (5.1.3.1) or dipotassium hydrogen phosphate solution (5.1.3.2) at pH 7,5 ± 0,2.

NOTE For better reconstitution and in particular with roller-dried milk, glass beads (6.4) can be helpful. If used, they are added to the bottle before sterilization.

Swirl slowly until the test portion has dispersed completely. Allow to stand for 5 min, swirling occasionally.

A peristaltic blender may be used, if dispersion is not complete.

The diluent may be pre-warmed to (44 to 47) °C in a water bath (6.1) if a homogeneous suspension cannot be obtained even after blending. Mention such an additional procedure in the test report.

9.3 Cheese and cheese products

Weigh the test portion into the container of a rotary blender or of a peristaltic blender. Add a diluent for general use (5.1.2), sodium citrate solution (5.1.3.1) or dipotassium hydrogen phosphate solution (5.1.3.2) at pH 7,5 ± 0,2. Blend until the cheese is thoroughly dispersed. Allow any foam to disperse.

The diluent may be pre-warmed to (44 to 47) °C in a water bath (6.1) if a homogeneous suspension cannot be obtained even after blending. Mention such an additional procedure in the test report.

9.4 Acid casein, lactic casein, rennet casein and caseinate

9.4.1 General case

Thoroughly mix the contents of the closed container by repeatedly shaking and inverting it.

Weigh the test portion into a sterile plastic bag for a peristaltic blender. Add the appropriate diluent at room temperature, as follows:

a) for acid and lactic casein: dilute with dipotassium hydrogen phosphate solution with antifoam agent (5.1.3.3) at pH 8,4 ± 0,2;

b) for caseinate: dilute with peptone-salt solution (5.1.2), sodium citrate solution (5.1.3.1) or dipotassium hydrogen phosphate solution (5.1.3.2) at pH 7,5 ± 0,2;

c) for rennet casein: dilute with dipotassium hydrogen phosphate solution with antifoam agent (5.1.3.3) at pH 7,5 ± 0,2.

Mix well manually and allow to stand at room temperature for 15 min. Blend for 2 min in the peristaltic blender by using, if necessary, two sterile bags for granular products. Allow to stand for 5 min.

9.4.2 Special case: Rennet casein

Rennet casein can be difficult to dissolve. An alternative procedure to that described in 9.4.1 may be used.

Using dipotassium hydrogen phosphate solution with antifoam agent (5.1.3.3) as diluent for rennet caseins may not be efficient to dissolve the grains. These casein grains hamper the enumeration of microorganisms at 30 °C. Therefore, the following alternative procedure is recommended.

If necessary, grind the dry casein before taking the test portion. Transfer approximately 20 g of the test sample into a suitable container. Grind it using an apparatus with blades able to rotate at approximately 20 000 r/min and equipped with a device that prevents the sample from heating during grinding[1)].

Weigh 5 g of the thus-prepared test sample in a sterile bottle of 250 ml with glass beads (6.4) to facilitate mixing. Add 95 ml of the sodium tripolyphosphate solution (5.1.3.4) preheated to (34 to 38) °C in a water bath (6.1). Mix by leaving the bottle on a mixing device for 15 min. Then, place it in the water bath (6.1) set at (34 to 38) °C for 15 min while mixing from time to time.

9.5 Butter

If it is necessary to exclude the surface of a butter sample from investigation, use a sterile spatula (6.2) to remove the upper layer on a thickness of at least 5 mm (see ISO 707 | IDF 50).

1) The VirTis apparatus is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.